Plant Physiol. (1980) 65, 541-543
0032-0889/80/65/0541/03$00.50/0

Nutrient Influences on Leaf Photosynthesis

EFFECTS OF NITROGEN, PHOSPHORUS, AND POTASSIUM FOR *GOSSYPIUM HIRSUTUM* L.[1]

Received for publication July 16, 1979 and in revised form October 12, 1979

DAVID J. LONGSTRETH[2] AND PARK S. NOBEL
Department of Biology and Division of Environmental Biology of the Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los Angeles, California 90024

ABSTRACT

The net rate of CO_2 uptake for leaves of *Gossypium hirsutum* L. was reduced when the plants were grown at low concentrations of NO_3^-, PO_4^{2-}, or K^+. The water vapor conductance was relatively constant for all nutrient levels, indicating little effect on stomatal response. Although leaves under nutrient stress tended to be lower in chlorophyll and thinner, the ratio of mesophyll surface area to leaf area did not change appreciably. Thus, the reduction in CO_2 uptake rate at low nutrient levels was due to a decrease in the CO_2 conductance expressed per unit mesophyll cell wall area ($g^{cell}_{CO_2}$). The use of $g^{cell}_{CO_2}$ and nutrient levels expressed per unit of mesophyll cell wall provides a new means of assessing nutrient effects on CO_2 uptake of leaves.

Plant mineral status can markedly affect photosynthesis. For instance, J_{CO_2}[3] is reduced in leaves that are deficient in N (10, 16, 18, 25), P (16, 20, 22), or K (3, 16, 17, 21, 24). Describing J_{CO_2} as a diffusion process controlled by g_{wv} and $g^{mes}_{CO_2}$ (8) facilitates examination of nutrient effects on photosynthesis. With decreasing N content, $g^{mes}_{CO_2}$ appears to be the main control of J_{CO_2} in C_3 plants (11, 18). For sugar beet and clover, $g^{mes}_{CO_2}$ is more responsive to decreasing P or K than is g_{wv} (17, 18, 21).

Measurement of leaf anatomy allows division of $g^{mes}_{CO_2}$ into a geometrical component, A^{mes}/A, and $g^{cell}_{CO_2}$. The cellular term includes the diffusion pathway into the mesophyll cells, as well as the initial biochemical step of CO_2 fixation (14). Nutrient status could affect A^{mes}/A, since packing of mesophyll cells (6), the amount of cell wall per unit leaf dry weight (19), specific leaf weight (16, 25), and leaf thickness (2) have all been shown to vary with N and/or P levels. Nutrient effects on $g^{cell}_{CO_2}$ are also probable, since reductions in total soluble protein and total Chl are correlated with low N levels (12). The objective of the present study was to examine the specific effects of N, P, and K on A^{mes}/A and $g^{cell}_{CO_2}$ for cotton.

MATERIALS AND METHODS

Seedlings of *Gossypium hirsutum* L. var. Acala SJ-2 were transferred to hydroponic culture 12-14 days after germination. The concentrations of NO_3^-, PO_4^{2-}, and K^+ were individually varied from one sixty-fourth to four times their concentration in full strength Hoagland No. 1 solution (with Hoagland minor solution and 0.08 meq l^{-1} Fe^{2+} sequestered with EDTA; ref. 7). Two seedlings were grown per 8-liter container in growth chambers using a 12-h day at 26 C with 375 ± 50 μE m^{-2} s^{-1} PAR (provided by cool-white fluorescent lamps supplemented 8% with incandescent lights) and a 12-h night at 21 C.

Solution concentrations were expressed relative to Hoagland solution No. 1, which contains 16 meq l^{-1} NO_3^-, 2 meq l^{-1} PO_4^{2-}, and 4 meq l^{-1} K^+. For NO_3^- variation, the solution was modified by omitting KNO_3, varying the concentration of $Ca(NO_3)_2$, adding 2.5 meq l^{-1} K^+ as K_2SO_4, and adding $CaCl_2 \cdot 2\,H_2O$ as needed to give a minimum Ca^{2+} concentration of 5 meq l^{-1}. For PO_4^{2-} variation, the KH_2PO_4 concentration was changed. For K^+ variation, KH_2PO_4 was omitted, K_2SO_4 was varied, and 2 meq l^{-1} PO_4^{2-} as $Ca(H_2PO_4)_2$ was added. The range in solution osmotic potential from low to high NO_3^-, PO_4^{2-}, and K^+ averaged 0.17 MPa, which has little effect on morphology and photosynthetic response of cotton (9).

Gas exchange and anatomical measurements were made on the third or fourth leaf above the cotyledonary leaves. These were mature leaves that had developed under a given nutrient treatment for 21-28 days. Rates of water vapor loss and J_{CO_2} were determined at 2,000 ± 100 μE m^{-2} s^{-1} PAR (light saturation) on attached leaves of at least two plants from each nutrient using a null-point closed-circuit flow system with circulating air containing approximately 1% O_2 (15). The low O_2 minimized effects of respiration and photorespiration. Leaf temperature was maintained at 30 ± 1 C, and the water vapor pressure difference between leaf and air was 1.6 ± 0.2 kPa.

Gas exchange rates were analyzed using the appropriate conductances (8, 14). The transpiration rate divided by the water vapor concentration drop from leaf to air gave g_{wv} (8). J_{CO_2} was plotted *versus* the CO_2 concentration in the intercellular air spaces next to the stomates, which was the CO_2 concentration outside the leaf minus 1.56 J_{CO_2}/g_{wv}; the slope of the line connecting the CO_2 compensation point and the J_{CO_2} value at ambient CO_2 concentration (340 μl l^{-1}) was designated $g^{mes}_{CO_2}$ (9, 14). Individual J_{CO_2} values varied less than 10% from the reported means. To determine A^{mes}/A, fresh sections cut from the side of the leaf midvein were infiltrated with distilled H_2O and examined using a Zeiss microscope with a camera lucida. Cell surface areas were calculated assuming that palisade cells were cylindrical with hemispherical ends and spongy cells were spheres (13). Using A^{mes}/A and $g^{mes}_{CO_2}$, $g^{cell}_{CO_2}$ was calculated:

$$g^{cell}_{CO_2} = g^{cell}_{CO_2}/(A^{mes}/A)$$

Tissue NO_3^- concentration was measured with a nitrate ion electrode (Orion 92-07) on samples ground in 25 mM $Al_2(SO_4)_3$

[1] This investigation was supported by National Science Foundation Grant DEB 77-11128.

[2] Present address: Department of Botany, Louisiana State University, Baton Rouge, Louisiana 70803.

[3] Abbreviations: J_{CO_2}: net CO_2 exchange rate per unit leaf area; g_{wv}: water vapor conductance (primarily stomatal); $g^{mes}_{CO_2}$: CO_2 mesophyll conductance; A^{mes}/A: surface area of mesophyll cells per unit leaf surface area; $g^{cell}_{CO_2}$: cellular CO_2 conductance expressed on a mesophyll surface area basis.

(5). Total P and K was measured using emission spectrography (23). To express NO_3^-, P, and K per unit mesophyll cell surface area, the amounts per unit leaf surface area were divided by A^{mes}/A. Chl was measured on 80% acetone extracts (1), and soluble protein using a Bio-Rad protein assay (4). Approximately 50 cm^2 leaf tissue, measured with a Lambda LI-3000 leaf area meter, was dried to constant weight at 60 C for determination of specific leaf weight (mg dry weight/cm^2 leaf area).

RESULTS

The hydroponic treatments produced a wide range of leaf nutrient levels. Leaf NO_3^--N ranged from 0.02 to 0.55% (dry weight/dry weight) from the low to high NO_3^- treatment, leaf P ranged from 0.05 to 1.34% for the PO_4^{2-} treatments, and K^+ ranged from 0.16 to 2.97% for the K^+ treatments. Total Chl was reduced 50% in the low NO_3^- and K^+ treatments, but only about 8% in low PO_4^{2-} (Table I). Specific leaf weights for all leaves used in J_{CO_2} measurements averaged 4.4 mg cm^{-2}, 10–20% higher values occurring at the lower nutrient levels. Soluble protein/cm^2 leaf area (assayed for the NO_3^- treatment only) increased 300% from one sixty-fourth to one-fourth strength NO_3^- and remained constant at higher concentrations.

Nutrient treatment did not affect epidermal thickness (upper surfaces averaged 15 μm and lower surfaces, 16 μm), and so differences in leaf thickness mirrored differences in mesophyll thickness. For the lowest nutrient concentrations the mesophyll region was 8–12% thinner than the average value (Table 1). However, A^{mes}/A was relatively constant for all treatments (Fig. 1).

At the lowest nutrient concentrations, J_{CO_2} was approximately 50% of the value found at one-fourth strength Hoagland solution (Fig. 2A). Stomatal conductance (indicated by g_{wv}) changed little with increasing concentration of each nutrient (Fig. 2B). The increase in J_{CO_2} with increasing nutrient levels reflected a greater $g^{mes}_{CO_2}$ (Fig. 2C), which in turn was due to changes in $g^{cell}_{CO_2}$. In fact, $g^{cell}_{CO_2}$ more than doubled from the low to one-fourth strength NO_3^-, PO_4^{2-}, and K^+ (Fig. 3). For each nutrient, $g^{cell}_{CO_2}$ rose rapidly at low nutrient levels (expressed as amount per unit of mesophyll cell surface) and then remained constant at about 0.15 mm s^{-1} over a large range (Fig. 4).

DISCUSSION

Reduction in the net rate of CO_2 uptake occurred at low concentrations of NO_3^-, PO_4^{2-}, and K^+ for cotton (Fig. 2). The reduction was primarily due to a decrease in $g^{mes}_{CO_2}$, similar to findings for nutrient effects on other species (11, 18, 20, 21). Cell dimensions decreased slightly at the lowest nutrient levels, but in such a way that A^{mes}/A varied little (Fig. 1). Thus, nutrient effects on J_{CO_2} reflected changes at the cellular level as represented by $g^{cell}_{CO_2}$ (Fig. 3), similar to previous results on *Plectranthus parviflorus* (13). Such responses are in contrast to those for differences in illumination during leaf development, which affects primarily A^{mes}/A (13, 14), and salinity, which can affect both A^{mes}/A and $g^{cell}_{CO_2}$ (9).

Changes in stomatal conductance (deduced by measuring g_{wv}) did not appreciably affect J_{CO_2} at any nutrient concentration (Fig. 2). Although previous work with C_3 plants indicated that variations in NO_3^- mainly influenced $g^{mes}_{CO_2}$ (11, 18), appreciable changes

Table I. *Total Chl and Leaf Thickness for Plants Grown at Various Concentrations of NO_3^-, PO_4^{2-}, or K^+*

SE of means averaged 4% for Chl ($n = 6$) and 5% for mesophyll thickness ($n = 10$ or 12).

	Solution Concentration (Hoagland units)				
	1/64	1/16	1/4	1	4
Total Chl (μg cm^{-2})					
NO_3^-	21	39	48	46	49
PO_4^{2-}	42	50	46	47	51
K^+	26	48	46	50	46
Mesophyll thickness (μm)					
NO_3^-	198	214	211	223	232
PO_4^{2-}	208	214	220	242	226
K^+	200	228	240	240	224

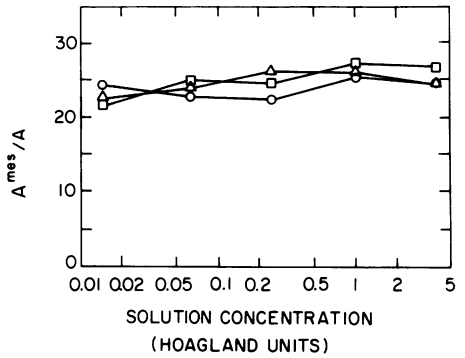

FIG. 1. A^{mes}/A from leaves of cotton grown hydroponically under various concentrations of NO_3^- (○), PO_4^{2-} (△), or K^+ (□).

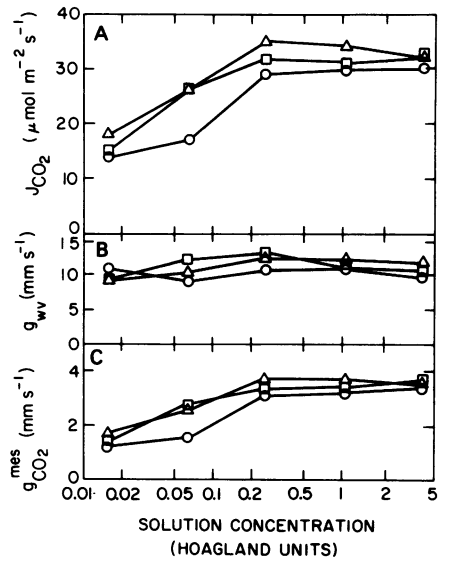

FIG. 2. J_{CO_2} and related conductances, g_{wv}, $g^{mes}_{CO_2}$, for plants grown under various concentrations of NO_3^- (○), PO_4^{2-} (△), or K^+ (□).

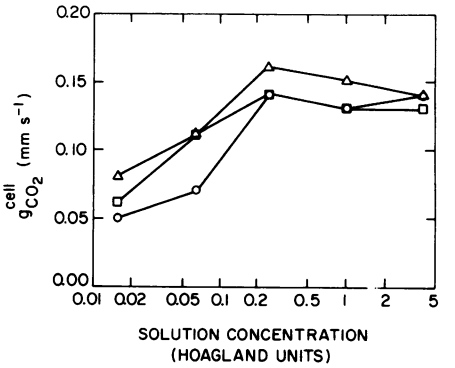

FIG. 3. $g^{cell}_{CO_2}$ for plants grown under various concentrations of NO_3^- (○), PO_4^{2-} (△), or K^+ (□).

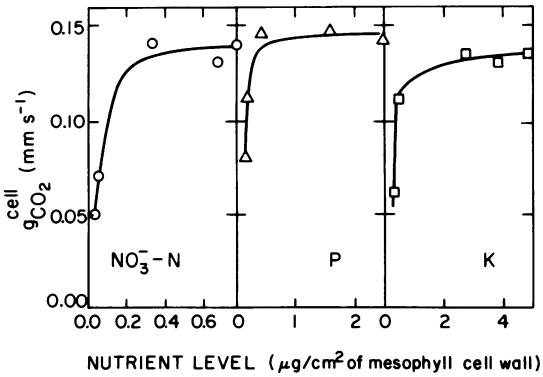

FIG. 4. $g^{cell}_{CO_2}$ at different nutrient levels per unit area of mesophyll cell wall.

in g_{wv} can occur. Terry and Ulrich (20, 21) showed that g_{wv} declined under PO_4^{2-} and K^+ stress, but this occurred after declines in $g^{mes}_{CO_2}$ had substantially lowered J_{CO_2}. Likewise, as K^+ deficiency increased in *Medicago sativa*, $g^{mes}_{CO_2}$ responded before g_{wv} (17). Therefore, changes in g_{wv} may be a secondary response to nutrient stress.

Although A^{mes}/A varied little (Fig. 1), low nutrient levels had an effect on several leaf properties. Cotton leaves from plants developing under nutrient stress were thinner than those developing under normal concentrations (Table I), unlike two Australian tree species, in which leaf thickness increased with N and P stress (2). Similar to wheat (16), but contrary to rice (25), specific leaf weight of cotton was highest at the lowest NO_3^- concentration (as well as lowest PO_4^{2-} and K^+ for cotton). As in previous reports (11, 12), total Chl was reduced in low NO_3^-, low K^+, and to a lesser extent low PO_4^{2-}; soluble protein was reduced in low NO_3^-. Our results suggest that some chemical component related to the photochemistry or biochemistry of photosynthesis was leading to the lower $g^{cell}_{CO_2}$ under low nutrient treatments.

Relating $g^{cell}_{CO_2}$, which varied more with nutrient treatment than the other factors controlling J_{CO_2}, to nutrient level per unit cell surface area (Fig. 4) provides a direct assessment of the nutrient level necessary for maximum $g^{cell}_{CO_2}$. The lower the level necessary to produce a maximum $g^{cell}_{CO_2}$, the better potential there is for adaptation of the plant to low nutrient values. This type of comparison could be used to quantify adaptation to nutrient stress of different species or to screen genotypes of crop species for use in nutrient-poor soil.

Acknowledgments—We thank Dr. Wade L. Berry for discussions on nutrient effects, and Mr. Terry L. Hartsock for gas exchange and NO_3^- measurements.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenol-oxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
2. BEADLE NCW 1968 Some aspects of the ecology and physiology of Australian xeromorphic plants. Aust J Sci 30: 348–355
3. BERSHTEIN BI, SY IVANISCHEVA, EM EL'YASHCHUK, IL BELOUS, AK PSHENICHNAYA, AS OKANENKO 1971 Photosynthesis, respiration, and phosphorus metabolism in plants in connection with potassium distribution under K-deficit conditions. Sov Plant Physiol 18: 436–442
4. BRADFORD MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254
5. CANTLIFFE DJ, GE MACDONALD, NH PECK 1970 The potentiometric determination of nitrate and chloride in plant tissue. NY Food Life Sci Bull 1 (3): 1–7
6. CHONON N 1970 Studies on the photosynthetic tissues in the leaves of cereal crops. VI. Effect of nitrogen nutrition on the mesophyll structure of leaves in rice varieties of different plant types. Proc Crop Sci Soc Japan 39: 426–430
7. HOAGLAND DR, DI ARNON 1950 The water-culture method for growing plants without soil. Calif Agric Exp Sta Circ 347: 1–32
8. JARVIS PG 1971 The estimation of resistances to carbon dioxide transfer. *In* Z Sestak, J Catsky, PG Jarvis, eds, Plant Photosynthetic Production: Manual of Methods. Dr W Junk, The Hague, pp 566–622
9. LONGSTRETH DJ, PS NOBEL 1979 Salinity effects on leaf anatomy. Consequences for photosynthesis. Plant Physiol 63: 700–703
10. MEDINA E 1971 Relationships between nitrogen level, photosynthetic capacity, and carboxydismutase activity in *Atriplex patula* leaves. Carnegie Inst Wash Year Book 69: 655–662
11. NATR L 1972 Influence of mineral nutrients on photosynthesis of higher plants. Photosynthetica 6: 80–99
12. NEVINS DJ, RS LOOMIS 1970 Nitrogen nutrition and photosynthesis in sugar beet (*Beta vulgaris* L.). Crop Sci 10: 21–25
13. NOVEL PS 1977 Internal leaf area and cellular CO_2 resistance: photosynthetic implications of variations with growth conditions and plant species. Physiol Plant 40: 137–144
14. NOBEL PS 1980 Leaf anatomy and water-use efficiency. *In* NC Turner, PJ Kramer, eds, Adaptation of Plants to Water and High Temperature Stress. John Wiley & Sons, New York. In press
15. NOBEL PS, TL HARTSOCK 1978 Resistance analysis of nocturnal carbon dioxide uptake by a crassulacean acid metabolism succulent, *Agave deserti*. Plant Physiol 61: 510–514
16. OXMAN AM, PJ GOODMAN, JP COOPER 1977 The effects of nitrogen, phosphorus and potassium on rates of growth and photosynthesis of wheat. Photosynthetica 11: 66–75
17. PEOPLES TR, DW KOCH 1979 Role of potassium in carbon dioxide assimilation in *Medicago sativa* L. Plant Physiol 63: 878–881
18. RYLE GJA, JD HESKETH 1969 Carbon dioxide uptake in nitrogen-deficient plants. Crop Sci 9: 451–454
19. SHIMSHI D 1970 The effect of nitrogen supply on some indices of plant-water relations of beans (*Phaseolus vulgaris* L.). New Phytol 69: 413–424
20. TERRY N, A ULRICH 1973 Effects of phosphorus deficiency on the photosynthesis and respiration of leaves of sugar beet. Plant Physiol 51: 43–47
21. TERRY N, A ULRICH 1973 Effects of potassium deficiency on the photosynthesis and respiration of leaves of sugar beet. Plant Physiol 51: 783–786
22. THYOK NT, TA AVDEEVA, TF ANDREEVA 1973 Effect of phosphorus nutrition on activity of the photosynthesis apparatus in leaves of different tiers of bean plants. Sov Plant Physiol 20: 871–874
23. WALLACE A, EM ROMNEY, GV ALEXANDER 1974 Variation in the simultaneous analysis by emission spectrography of twenty-four elements in plant material. Commun Soil Sci Plant Anal 5: 45–50
24. WOLF DD, EL KIMBROUGH, RE BLASER 1976 Photosynthetic efficiency of alfalfa with increasing potassium nutrition. Crop Sci 16: 292–294
25. YOSHIDA S, V CORONEL 1976 Nitrogen nutrition, leaf resistance, and photosynthetic rate of the rice plant. Soil Sci Plant Nutr 22: 207–211